Age related changes in the interconversion of inositol phospholipids in the rat brain cortex

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AGE-RELATED CHANGES IN THE INTERCONVERSION OF INOSITOL PHOSPHOLIPIDS IN RAT BRAIN CORTEX

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ABSTRACT

Specific phosphate incorporation activities into PIP and PIP_2 in a lysed crude synaptosomal fraction of rat brain cortex showed no age related changes. Specific phosphate incorporation into PA, however, decreased with age. The total phosphate incorporation activities into PIP, PIP_2 and PA in the lysed crude synaptosomal fraction of rat brain cortex also decreased with age which could be caused by an age related loss of active nerve endings.

INTRODUCTION

Inositol phospholipids which are relatively enriched in brain tissue play very important physiological roles such as the regulation of membrane permeability, calcium binding to the membrane, and the conversion of extracellular signals into intracellular signals (Berridge, 1987). Findings such as age related changes in inositol phospholipid concentrations (Stokes et al., 1983) which are probably correlated to changes in membrane fluidity (Schroeder, 1988) support the idea that the interconversion of these inositol phospholipids in the brain changes during aging, but strikingly there have been no studies on this subject. The present study was designed to provide some information on age related changes in phosphate incorporation activities into PIP (phosphatidylinositol-4-phosphate), PIP_2 (phosphatidylinositol-4,5-biphosphate) and PA (phosphatidic acid) in a crude synaptosomal fraction of the rat brain cortex.

EXPERIMENTAL PROCEDURES

Brain dissection: Male Wistar rats (7 and 27 months old) were decapitated, whereafter the head was immediately immersed in liquid nitrogen for 8 sec. The dissection of the brain cortex was performed at 0-4°C.
Subcellular fractionation: Tissue from individual rats was homogenized (medium: 0.32 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) in a total volume 10 times the tissue volume.The homogenate was centrifuged for 10

min at 1000 g. After centrifugation of the supernatant at 10,000 g for 10 min, the resulting crude mitochondrial/synaptosomal pellet (P_2) was subjected to osmotic lysis (20 min) by resuspendation in 10 vol. aqua bidest at 4°C. This fraction was taken as the enzyme fraction (stored at -80°C).

Phosphorylation assay: The assay for endogenous phosphate incorporation into PIP, PIP_2 and PA, lipid extraction and TLC were performed as described previously (Jolles et al., 1981; Bothmer, 1990). ^{32}P was determined by liquid scintillation counting (Ready Safe, Beckman).

RESULTS

The present study was performed to investigate if age has an effect on endogeneous phospholipid phosphorylation activities in rat brain cortex. Wistar rats of 7 months were compared with 27-month-old rats with regard to the effect of the duration of preincubation on the phosphate incorporation into PIP, PIP_2 and PA in a lysed crude synaptosomal fraction of brain cortex. A previous methodological study (Bothmer et al.,1990) revealed that increasing preincubation times markedly decreased the phosphate incorporation into PIP_2, whereas the phosphate incorporation into PIP and PA were not altered.

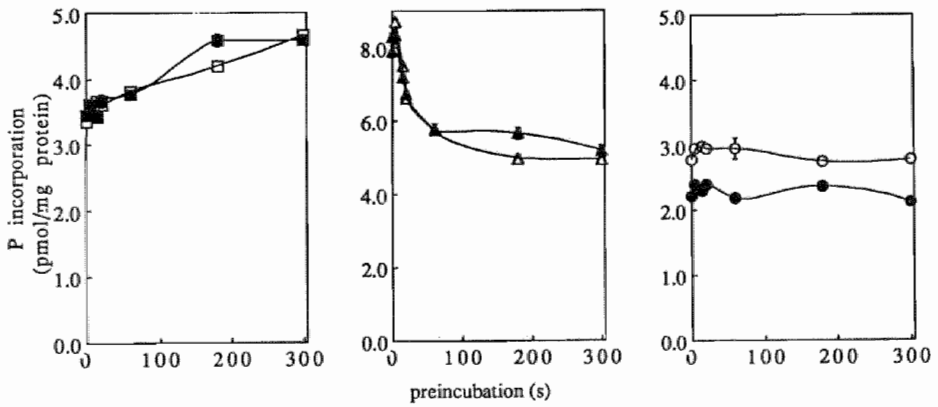

Fig. 1 Effect of preincubation on the incorporation of phosphate (pmol/mg protein) into PIP (squares), PIP_2 (triangles) and PA (circles) in a lysed crude synaptosomal fraction of rat brain cortex of 7-month-old (open symbols) and 27-month-old (closed symbols) rats. (mean $\pm$ SEM ; N=5).

Fig. 1 shows that there is no effect of age on the specific activities of phosphate incorporation into PIP and PIP_2, but phosphate incorporation into PA appeared to be decreased in 27-month-old rats. The increase in phosphate incorporation into PIP with longer preincubation times seems in contrast with a previous study (Bothmer et al., 1990) in which phosphate incorporation into PIP was not affected by changing preincubation times. However, they used a crude synaptosomal fraction in which unlysed structures were removed. The present study was performed without the removal of these unlysed structures.

The protein concentration in the enzyme fraction of 27-month-old rats was decreased with 14.5% compared with 7-month-old rats. Because of this, the total phosphate incorporation activities into PIP, PIP_2 and PA in the lysed crude synaptosomal fraction of 27-month-old rat brain cortex are decreased when compared with 7-month-old rats (Fig. 2).

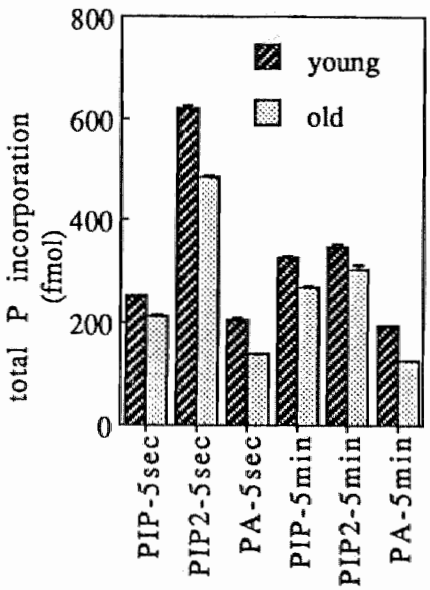

Fig. 2 Total phosphate (P) incorporation rates (fmol/10 μl crude synaptosomal fraction) into PIP, PIP_2 and PA in a lysed crude synaptosomal fraction of rat brain cortex of 7-month-old (young) and 27-month-old (old) rats after 5 sec or 5 min preincubation.

DISCUSSION

The changes in total phosphate incorporation activities into PIP, PIP_2 and PA in the enzyme fraction used here, could be caused by a factor like myelinisation which changes during aging and influences the subcellular fractionation, but may also be caused by a loss of active nerve endings in the rat brain cortex during aging. Further studies will be performed on this subject but also subcellular and regional differences in phosphate incorporation activities in inositol phospholipids will be studied. After these localization studies, aging studies will be performed in which more groups of animals differing in age are used.

REFERENCES

Berridge M.J., Inositol triphosphate and diacylglycerol: Two interacting second messengers, Annu. Rev. Biochem. 56 (1987) 159-193.

Bothmer J., M. Markerink and J. Jolles, Phosphatidic acid and polyphosphoinositide formation in a broken cell preparation from rat brain: effects of different incubation conditions, Neurochem. Int. 17 (1990) 27-33.

Jolles J., H. Zwiers, A. Dekker, K.W.A. Wirtz and W.H. Gispen, Corticotropin-(1-24)-tetracosapeptide effects protein phosphorylation and polyphosphoinositide metabolism in rat brain, Biochem. J. 194 (1981) 283-291.

Schroeder F., Synaptosomal plasma membrane (spm) lipid and structural asymmetry, Proceedings of the Guiseppe Porcellati Foundation, Int. Meeting on Neurochem. Aspects of phospholipid metabolism, May 1988, Perugia (It.).

Stokes C.E., K.R.W. Gillon and J.N. Hawthorne, Free and total lipid myoinositol concentration decrease with age in human brain, Biochim. Biophys. Acta 753 (1983) 136-138.